

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
July 15, 2014**

Cover Page

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Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0206-9-073
USDA-ARS Agreement Title:	Molecular Genetic Approaches to Develop Scab Resistance.
FY13 USDA-ARS Award Amount:	\$ 140,111

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Next Generation Approaches to Characterize Barley FHB Resistant QTL.	\$ 73,025
GDER	Rapidly Identify and Test Scab Resistance Genes.	\$ 67,086
	FY13 Total ARS Award Amount	\$ 140,111

Gary J. Muehlbauer

July 10, 2014

Principal Investigator

Date

* MGMT – FHB Management
 FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
 GDER – Gene Discovery & Engineering Resistance
 PBG – Pathogen Biology & Genetics
 BAR-CP – Barley Coordinated Project
 DUR-CP – Durum Coordinated Project
 HWW-CP – Hard Winter Wheat Coordinated Project
 VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
 SPR – Spring Wheat Region
 NWW – Northern Soft Winter Wheat Region
 SWW – Southern Soft Red Winter Wheat Region

Project 1: *Next Generation Approaches to Characterize Barley FHB Resistant QTL.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Fusarium head blight (FHB or scab), caused by *Fusarium graminearum*, is a disease that can devastate the small grains wheat and barley. There is a limited amount of information pertaining to the molecular genetic interaction between barley and *F. graminearum*. Our goal is to identify, characterize and map barley genes that respond to *F. graminearum* infection.

We have initiated an experiment to examine gene expression at key QTL for FHB resistance. We are using three near-isogenic line (NIL) pairs carrying resistant and susceptible alleles for FHB resistant QTL at chromosome 2H bin 8 and 10, and chromosome 6H bin 7. To genetically characterize these NILs, we collaborated with Shiaoan Chao (USDA-ARS, Fargo, ND) to SNP genotype the lines with a 9K SNP platform. The SNP analysis showed that the lines were highly isogenic. We inoculated pikes from the three pairs of NILs with *F. graminearum* and mock water and sampled spikes at 48 and 96 hours after inoculation. There are a total of 72 samples (six genotypes, two treatments, two timepoints and three replications) that we isolated RNA from. RNA-seq data has been obtained and the analysis has been initiated. We have identified differentially expressed barley and *F. graminearum* genes between the resistant and susceptible alleles for each QTL. The differentially expressed genes have been mapped to the barley genome. Additional analysis is ongoing. Disease severity, deoxynivalenol (DON) and ergosterol data have been obtained from the samples used for the RNA isolation. We also obtained FHB severity, DON and ergosterol readings from the lines in a replicated trial in the field in 2013 and another trial is currently being conducted in the field in 2014.

Additional mapping work is also being initiated. To conduct fine mapping of the chromosome 6H bin 7 and chromosome 2H bin 8 QTL, we initiated population development by crossing near-isogenic lines carrying the resistant or susceptible alleles for each locus. Crosses were made and the F1s are being grown to develop populations for mapping. We are also developing recombinant inbred line populations from crosses between highly susceptible barley genotypes and moderately susceptible lines. These populations will be used to detect minor effect QTL for FHB resistance that are not detectable due to detecting the major effect genes when using a resistant genotype as one of the parents.

In collaboration with Brian Steffenson and Kevin Smith we identified a set of 78 barley genotypes carrying resistance to *F. graminearum*. These 78 resistant lines along with 23 susceptible lines were screened with DArT markers and we determined the haplotypes at three FHB QTL on chromosome 2H bins 8, 10 and 13-14 and a QTL on chromosome 6H bin 7 (Huang et al., 2012). This information will be useful for future mapping studies and breeding efforts. We also used the DArT data to identify a core set of genotypes that serve as a representative set for sequencing genes that have the potential to exhibit resistance or susceptibility. From previous microarray experiments we identified 39 genes that were induced during infection. We completed the sequencing of these 39 genes from a core set of

24 genotypes. Also, as part of this sequencing effort, we completely sequenced a barley UDP-glucosyltransferase (HvUGT13248; see below) gene (~2.5 kb), which detoxifies DON via conversion to DON-3-O-glucoside, from 40 individuals. Upon close inspection of the haplotypes from this sequencing effort, none of these genes showed a clear association with resistance or susceptibility.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

Accomplishment:

1. We established a large RNA-seq experiment to study gene expression in the host (barley) and pathogen (*F. graminearum*) during infection. Analysis of the RNA-seq data is ongoing. We complemented the RNA-seq data with disease severity, DON and ergosterol data from growth chamber and field grown plants.
2. We determined DArT haplotypes in 101 FHB resistant and susceptible genotypes for four major barley FHB resistant QTL. We published a paper in Theoretical and Applied Genetics (Huang et al., 2012)
3. We determined that the haplotypes for 39 barley genes that are induced by *F. graminearum* are not associated with resistance or susceptibility.
4. Mapping populations for identifying novel FHB resistant QTL and fine mapping previously identified QTL are in development.

Impact:

1. The DArT haplotype work of the four FHB resistant QTL provided information regarding the genetic relationships between the FHB resistant and susceptible genotypes, enabling breeders to select the genotypes that carry novel disease resistance loci and/or alleles for genetic mapping and breeding.
2. The RNA-seq data is providing a rich resource for additional mapping of FHB resistant QTL and gene discovery in barley for FHB resistance genes.

Project 2: *Rapidly Identify and Test Scab Resistance Genes.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Fusarium head blight (FHB or scab), caused by *Fusarium graminearum*, is a disease that can devastate the small grains wheat and barley. During infection, *F. graminearum* produces deoxynivalenol (DON), a trichothecene mycotoxin. There is a limited amount of information pertaining to the molecular genetic interaction between the small grains and *Fusarium graminearum* and in particular the response to DON accumulation. Our goal is to identify and rapidly test genes that exhibit resistance to trichothecenes.

Six and four transgenic wheat lines carrying the barley UDP-glucosyltransferase gene (HvUGT13248) were developed in the Bobwhite and CB037 backgrounds, respectively. The Bobwhite and CB037 transgenics were screened in the greenhouse three and two times, respectively. In the greenhouse screens, the transgenic lines exhibited significantly reduced disease severity often times reaching the equivalent levels of Sumai3. In collaboration with Dr. Ruth Dill-Macky, field screens were conducted in 2012 and 2013 and lines were identified that exhibited significantly reduced FHB severity, very scabby kernels (VSK), and DON concentration. The level of FHB resistance that was observed was similar to Sumai3. Another field screen has been established in 2014. We collaborated with Franz Berthiller (University of Natural Resources and Life Sciences, Austria) to show that the mechanism of resistance in the HvUGT1328 wheat transgenics is the conjugation of DON to DON-3-glucoside. We also showed that the wheat transgenics carrying HvUGT13248 exhibit resistance to a nivalenol-producing *F. graminearum* strain. To determine if HvUGT13248 introgressed into a type II genetic background will result in enhanced resistance, multiple Bobwhite transgenics were backcrossed to Rollag (type II resistant spring wheat derived from Fhb1) and BC1 populations derived carrying the transgene and Fhb1. These populations were screened in the greenhouse and no difference was detected between plants carrying the transgene and Fhb1 and the type II resistant controls. Additional testing of these lines is being conducted in the 2014 field screen. We also collaborated with Dr. Jochen Kumlehn (IPK-Gaterslaben, Germany) to develop transgenic barley overexpressing HvUGT13248. We have seven transgenic barley lines in the Golden Promise background that expressed the transgene and these lines exhibited resistance to DON. To screen the lines in the field, we developed BC1 lines in the Rasmusson genetic background. We plan to screen these lines in the field in 2015.

To rapidly identify additional DON resistance genes, we transformed Arabidopsis with putative DON resistance genes from barley. We transformed Arabidopsis with a zinc finger protein, two ABC transporters, two cytochrome P450s, one epoxide hydrolase, three glutathione-S-transferases and a cysteine synthase. To date, testing of the transgenic Arabidopsis has not resulted in the identification of DON resistant plants.

- 2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:**

Accomplishment:

1. We developed transgenic wheat and barley carrying a barley UDP-glucosyltransferase (HvUGT13248). In greenhouse and field screens, the wheat transgenics exhibited a high level of FHB resistance and DON accumulation. The barley transgenics exhibited resistance to DON.
2. We demonstrated that the mechanism of resistance conferred by HvUGT13248 is the conjugation of DON to DON-3-glucoside.
3. We showed that the wheat transgenics carrying HvUGT13248 exhibited resistance to nivalenol-producing *F. graminearum*.
4. We backcrossed the HvUGT13248 transgene into the Type II resistant cv. Rollag background, selected lines that contained both the HvUGT13248 transgene and Fhb1 and tested the lines in the greenhouse. In an initial greenhouse screen, these lines did not exhibit higher levels of resistance compared to the type II resistant control. Additional field tests are ongoing.

Impact:

1. The transgenic wheat carrying HvUGT13248 exhibited an equivalent level of resistance to Sumai3, indicating that this gene is useful to confer a high level of resistance in wheat.
2. The transgenic barley carrying HvUGT13248 exhibited a high level of resistance to DON, indicating that overexpressing the gene in barley will result in FHB resistance.
3. The demonstration of HvUGT13248 conjugating DON to DON-3-glucoside provides a key piece of information for future strategies to detoxify DON.
4. The demonstration of HvUGT13248 in transgenic wheat conferring resistance to NIV-producing *F. graminearum*, indicates that HvUGT13248 acts on a broad spectrum of trichothecene mycotoxins.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer-reviewed publications:

Schweiger, W., B. Steiner, C. Ametz, G. Siegwart, G. Wiesenberger, F. Berthiller, M. Lemmens, H. Jia, G. Adam, G. Muehlbauer, D. Kreil and H. Buerstmayr. 2013. Transcriptomic characterization of two major Fusarium resistance QTL, *Fhb1* and *Qfhs.ifa-5A*, identify novel candidate genes. *Mol. Plant Path.* 14:772-785.

Kovalsky Paris, M.P., W. Schweiger, C. Hametner, R. Stückler, G.J. Muehlbauer, E. Varga, R. Krska, F. Berthiller and G. Adam. 2014. Formation of the novel masked mycotoxin zearalenone-16-O-glucoside by engineered yeast expressing a barley UDP-glucosyltransferase and by zearalenone treated plant cells. *J. Agric. Food Chem.* 62: 1181-1189.

Abstracts (2013):

Huang, Y., L. Li, K.P. Smith and G.J. Muehlbauer. 2013. RNA sequencing analyses of two barley near-isogenic line pairs identify genes associated with resistance to Fusarium head blight. In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 69.

Koeritz, E.J., A.M. Elakkad, G.J. Muehlbauer, X. Li, L.S. Dahleen, T. Abebe, R.W. Skadsen and R. Dill-Macky. 2013. Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2013 field nursery report. In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. pp.70-71.

Li, X., S. Shin, S. Heinen, R. Dill-Macky, F. Berthiller, T. Clemente, S. McCormick and G.J. Muehlbauer. 2013. Transgenic wheat carrying a barley UDP-glucosyltransferase exhibits high levels of Fusarium head blight resistance by detoxifying trichothecenes. In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 73.

Kovalsky Paris, M.P., W. Schweiger, G. Wiesenberger, JA. Torres Acosta, H. Michlmayr, S. Newmister, M. Lemmens, A. Malachova, S. Shin, G. Muehlbauer, T. Weigl-Pollack, P. Fruhmann, H. Mikula, C. Hametner, B. Kluger, R. Schuhmacher, R. Krska, J. Fröhlich, I. Rayment, F. Berthiller, and G. Adam. 2013. *In planta* inactivation of Fusarium mycotoxins. European Fusarium Symposium Abstracts.

Schweiger, W., M.P. Kovalsky, H. Michlmayr, G. Wiesenberger, R. Stückler, C. Schmeitzl, A. Malachova, B. Kluger, R. Schuhmacher, R. Krska, M. Lemmens, H. Buerstmayr, P. Fruhmann, T. Weigl-Pollack, H. Mikula, C. Hametner, J. Fröhlich, G.J. Muehlbauer, S. Newmister, I.

FY13 (approx. May 13 – May 14)

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Rayment, F. Berthiller and G. Adam. 2013. UDP-glucosyltransferases: Resistance to *Fusarium* mycotoxins and formation of masked mycotoxins. In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 78.